

L3 ANSWER 15 OF 30 MEDLINE DUPLICATE 10
 AN 97223729 MEDLINE
 DN 97223729 PubMed ID: 9056205
 TI Product differentiation by analysis of DNA **melting curves** during the **polymerase chain reaction**.
 AU Ririe K M; Rasmussen R P; Wittwer C T
 CS Idaho Technology Inc., Idaho Falls 83402, USA.
 NC GM 51647 (NIGMS)
 SO ANALYTICAL BIOCHEMISTRY, (1997 Feb 15) 245 (2) 154-60.
 Journal code: ANK; 0370535. ISSN: 0003-2697.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 199705
 ED Entered STN: 19970523
 Last Updated on STN: 19970523
 Entered Medline: 19970515
 AB A microvolume fluorometer integrated with a thermal cycler was used to acquire DNA **melting curves** during **polymerase chain reaction** by fluorescence monitoring of the double-stranded DNA specific dye **SYBR Green I**. Plotting fluorescence as a function of temperature as the thermal cycler heats through the dissociation temperature of the product gives a DNA **melting curve**. The shape and position of this DNA **melting curve** are functions of the GC/AT ratio, length, and sequence and can be used to differentiate amplification products separated by less than 2 degrees C in melting temperature. Desired products can be distinguished from undesirable products, in many cases eliminating the need for gel electrophoresis. Analysis of **melting curves** can extend the dynamic range of initial template quantification when amplification is monitored with double-stranded DNA specific dyes. Complete amplification and analysis of products can be performed in less than 15 min.

L3 ANSWER 17 OF 30 MEDLINE
 AN 97148028 MEDLINE
 DN 97148028 PubMed ID: 8994660
 TI Continuous fluorescence monitoring of rapid cycle DNA amplification.
 AU Wittwer C T; Herrmann M G; Moss A A; Rasmussen R P
 CS Department of Pathology, University of Utah Medical School, Salt Lake City 84132, USA.. ctwittwer@mscc.med.utah.edu
 NC I R41 GM51647 (NIGMS)
 SO BIOTECHNIQUES, (1997 Jan) 22 (1) 130-1, 134-8.
 Journal code: AN3; 8306785. ISSN: 0736-6205.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 199703
 ED Entered STN: 19970407
 Last Updated on STN: 19970407
 Entered Medline: 19970325
 AB Rapid cycle DNA amplification was continuously monitored by three different fluorescence techniques. Fluorescence was monitored by (i) the double-strand-specific dye **SYBR Green I**, (ii) a decrease in fluorescein quenching by rhodamine after exonuclease cleavage of a dual-labeled hydrolysis probe and (iii) resonance energy transfer of fluorescein to Cy5 by adjacent hybridization probes. Fluorescence data acquired once per cycle provides rapid absolute quantification of initial template copy number. The sensitivity of **SYBR Green I** detection is limited by nonspecific product formation. Use of a single

exonuclease hydrolysis probe or two adjacent hybridization probes offers increasing levels of specificity. In contrast to fluorescence measurement once per cycle, continuous monitoring throughout each cycle monitors the temperature dependence of fluorescence. The cumulative, irreversible signal of hydrolysis probes can be distinguished easily from the temperature-dependent, reversible signal of hybridization probes. By using **SYBR Green I**, product denaturation, annealing and extension can be followed within each cycle. Substantial product-to-product annealing occurs during later amplification cycles, suggesting that product annealing is a major cause of the plateau effect. Continuous within-cycle monitoring allows rapid optimization of amplification conditions and should be particularly useful in developing new, standardized clinical assays.

L3 ANSWER 22 OF 30 MEDLINE DUPLICATE 13
 AN 96299093 MEDLINE
 DN 96299093 PubMed ID: 8660567
 TI A quantitative method of determining initial amounts of DNA by **polymerase chain** reaction cycle titration using digital imaging and a novel DNA stain.
 AU Becker A; Reith A; Napiwotzki J; Kadenbach B
 CS Fachbereich Chemie, Philipps-Universität, Marburg, D-35032, Germany.
 SO ANALYTICAL BIOCHEMISTRY, (1996 Jun 1) 237 (2) 204-7.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 199609
 ED Entered STN: 19961008
 Last Updated on STN: 19961008
 Entered Medline: 19960925
 AB A new nonradioactive method is described for quantitative determination of small amounts of DNA by **PCR**, exemplified with mitochondrial DNA. The method represents a combination of serial dilution **PCR** and kinetic **PCR** and avoids the use of radioactivity by applying the fluorescent dye **SYBR Green I**, allowing visualization of **PCR** amplified bands on agarose gels in a broad exponential range of **PCR** cycles. After recording agarose gel images with a video camera in a computer, the band intensities are processed with the NIH image program and analyzed by a new graphical method. This nonradioactive method allows calculation of small original amounts of specific DNA in samples at high accuracy.

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